







# Serum TWEAK levels in patients with obstructive sleep apnea syndrome

 Suleyman Emre Karakurt<sup>1</sup>,  Nurcan Kum<sup>1</sup>,  Mehmet Ali Cetin<sup>1</sup>,  Canan Topcuoglu<sup>2</sup>,  Turan Turhan<sup>2</sup>,  
 Haci Huseyin Dere<sup>1</sup>

<sup>1</sup>Department of Otorhinolaryngology, Head and Neck Surgery, Health Sciences University Ankara Numune Training and Research Hospital, Ankara, Turkey

<sup>2</sup>Department of Biochemistry, Health Sciences University Ankara Numune Training and Research Hospital, Ankara, Turkey

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## Abstract

**Aim:** The purpose of the study is to research the serum TWEAK levels in obstructive sleep apnea syndrome (OSAS) patients.

**Material and Methods:** Eighty-six subjects were admitted in the study, 59 being in the patient group and 27 in the control group. The patient group was further divided into subgroups of severe OSAS and mild-moderate OSAS. TWEAK levels between the groups were statistically compared. In addition, the correlation of sleep-related variables with TWEAK levels was investigated in the patient group.

**Results:** TWEAK levels were lower in the patient group than in the control group. When the correlation between TWEAK levels and sleep-related variables are examined, inversely correlation between TWEAK levels and apnea-hypopnea index, the percentage of total sleep time spent with oxygen saturation below 90% and oxygen desaturation index was found.

**Conclusion:** Serum TWEAK levels in OSAS patients and its relation with sleep variables showed for the first time that TWEAK may be a new inflammatory biomarker for OSAS.

**Keywords:** Low-grade inflammation; polysomnography; sleep apne; TWEAK

## INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a disease with recurrent upper respiratory tract obstruction and generally results in oxygen desaturations and sleep interruptions(1).It has been documented in various studies that its prevalence is 0.3% - 15.0%(2). About 7% of the males and 5% of the females are estimated to be affected by OSAS (3). The underlying pathophysiological processes have not been completely identified yet; however, recent studies support that elevated oxidative stress and low-grade systemic inflammation may possibly play a role (4). When compared with the normal population, risks for cardiovascular and cerebrovascular incidents are elevated in OSAS patients, which are also thought to be associated with systemic inflammation. As a result, there has been an increase in the number of studies focusing on the inflammatory biomarkers such as interleukin-8 or TNF-alpha as evidence of systemic inflammation in OSAS patients (5).

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a bioinflammatory cytokine (6).

This cytokine can be expressed in a multitude of cells or tissues including immune system, such as macrophages and dendritic cells, and skeletal muscle(6,7). This cytokine exerts various activities such as inflammation and programmed cell death (8). TWEAK levels have been studied in various patient groups including chronic inflammation, malignancies and autoimmune diseases (7, 9, 10). As far as we know, the study investigating TWEAK levels in OSAS has not been found in the literature. The purpose of the research is to investigate the serum TWEAK levels in OSAS patients.

## MATERIAL and METHODS

### Subjects

After the approval of the institutional review board, the study was started (E-16-813). The permission of the subjects was obtained.Eighty-six patients were admitted in the research. Any subjects having a chronic autoimmune or inflammatory condition, cardiovascular disease, renal disease or any history of a malignancy were excluded. The participants were classified into patient or control groups. The patient group was further divided into subgroups of

**Received:** 23.02.2020 **Accepted:** 21.08.2020 **Available online:** 23.09.2020

**Corresponding Author:** Suleyman Emre Karakurt, Department of Otorhinolaryngology, Head and Neck Surgery, Health Sciences University Ankara Numune Training and Research Hospital, Ankara, Turkey

**E-mail:** suleymanemrekarakurt@gmail.com

severe (apnea-hypopnea index  $\geq 30$ /h) and mild-moderate (apnea-hypopnea index = 5-30/h) OSAS cases as newly diagnosed by whole-night polysomnography; while the subjects with an apnea-hypopnea index (AHI) of  $< 5$ /h were included in the control group. Severe OSAS, mild to moderate OSAS and control groups were compared in terms of basic clinical features, polysomnography findings and TWEAK levels. The correlation between TWEAK levels and polysomnographic parameters was evaluated in the patient group.

### Sleep study

Each subject was performed a whole-night polysomnography using Alice PSG system (Philips Respironics, The Netherlands). Electroencephalogram, electromyogram, electrooculogram, nasal airflow, respiratory efforts, blood oxygen saturation and body position information were recorded. Scoring was done by anoto-rhino-laryngology specialist with a sleep disturbances and polysomnography certificate, using the rules by American Academy of Sleep Medicine.

### Biochemical study

Venous samples were taken in separator tubes and centrifuged at 1300 g for 10 min after completion of clotting. Examples were separated and kept at  $-80^{\circ}\text{C}$  until analyzed. TWEAK levels were evaluated utilizing a commercial enzyme-linked immunosorbent test (ELISA) (eBioscience, An AffymetrixCompany, Austria. REF: BMS2006INST, LOT: 14026804) following the protocols by the manufacturer. The detection range of the assay was 15.6-1000 pg/ml. The limit of detection was 9.7 pg/ml. Intra- and inter-assay precision rates were 7.9% and 9.2%, respectively. Insulin concentrations were measured on DXI 800 Unicel (Beckman Coulter Inc., USA) using the chemiluminescence immunoassay (CLIA) method. Serum glucose levels were evaluated by enzymatic method with Beckman Coulter AU 5800 (Beckman Coulter Inc., USA) autoanalyzer. HOMA-IR was counted by the following recipe:  $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting blood glucose (mg/dL)} / 405$ .

### Statistical analysis

The Shapiro-Wilk test was utilized to evaluate the normality of parameters. Data were presented as median with minimum-maximum range or numbers with percentage. The differences of the basic clinical characteristic parameters (Table 1) of the patient and control groups were evaluated by Mann-Whitney U test or chi-square test. The difference of the sleep characteristic parameters of the severe OSAS, mild-moderate OSAS and control group (Table 2) was evaluated with the Kruskal-Wallis test (for three groups), and then Mann-Whitney U test was used for comparisons within the two groups. Spearman rank correlation coefficient was computed to assess the correlation between serum TWEAK levels and sleep-related variables (Table 3).  $P < 0.05$  values were considered significant. SPSS software version 21.0 (SPSS Inc, Chicago, IL) was utilized for statistical analysis.

## RESULTS

The patient group consisted of 59 patients; the control group consisted of 27 patients. The median and minimum-maximum age values of the patient and control groups were 49 (28-75) and 48 (21-63), respectively (Table 1). The patient group consisted of 23 women and 36 men; the control group consisted of 13 women and 14 men (Table 1). The median and minimum-maximum body mass index (BMI) values of the patient and control groups were 29.8 (23.1-68) and 28.1 (19.8-34.2), respectively (Table 1). The median and minimum-maximum homeostasis model assessment of insulin resistance (HOMA-IR) values of the patient and control groups were determined as 2.41 (0.45-35.9) and 2.27 (1.04-21.8), respectively (Table 1). There was no statistically significant difference between the age values, gender distributions, BMI values and HOMA-IR values of the groups (0.312, 0.424, 0.180, 0.699, p values, respectively) (Table 1).

**Table 1. Basic clinical characteristics of the control and patient groups**

	Patient Group (n=59)	Control Group (n=27)	p-value
Age (years)*	49 (28-75)	48 (21-63)	0.312
Sex**	Female	23 (39%)	0.424
	Male	13 (48.1%)	
BMI (kg/m <sup>2</sup> )*	29.8 (23.1-68)	28.1(19.8-34.2)	0.180
HOMA-IR*	2.41 (0.45-35.9)	2.27 (1.04-21.8)	0.699

\* Values are presented median with minimum-maximum range  
 \*\* Values are presented number with percentage.  
 BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance

When the patient group was grouped as severe and mild-moderate OSAS, 31 patients constituted the severe OSAS group and 28 patients formed the mild-moderate OSAS group.

Severe OSAS, mild-moderate OSAS and control group median and minimum-maximum AHI values were 54.7 (30.3-109.5), 9.6 (5-25.6) and 2.1 (0.2-4.9), respectively (Table 2). Severe OSAS, mild-moderate OSAS and control group median and minimum-maximum minimum SPO<sub>2</sub> (%) values were 79 (43-89), 86 (66-92) and 87.6 (58-93), respectively (Table 2). Severe OSAS, mild-moderate OSAS and control group median and minimum-maximum total sleep time spent with oxygen saturation below 90% (TST <90%) values were 9.7 (0-90.7), 0.3 (0-15.4) and 0 (0-20), respectively (Table 2). Severe OSAS, mild-moderate OSAS and control group median and minimum-maximum oxygen desaturation index (ODI) values were 44.3 (10.8-117.1), 7.9 (2.7-23.9) and 0.3 (0-3.1), respectively.(Table 2). Severe OSAS, mild-moderate OSAS and control group median and minimum-maximum TWEAK values were 590.9 (109.2-993.6), 797.6 (116.1-998.8) and 916.2 (574.8-991.3), respectively (Table 2).

Severe OSAS group AHI, minimum SPO<sub>2</sub> (%), TST <90%, ODI and TWEAK values were statistically significantly higher than both mild-moderate OSAS and control group. (p<0.001) (Table 2). Mild-moderate OSAS group AHI, minimum SPO<sub>2</sub> (%), TST <90%, ODI and TWEAK values were significantly higher than the control group (p<0.001) (Table 2).

The correlation between the sleep-related variables and serum TWEAK levels in patient group is presented in Table 3. Briefly, TWEAK levels were revealed to be inversely correlated with AHI, TST < 90% and ODI, while positively correlated with minimum SPO<sub>2</sub> (%) (p<0.001, r=-0.41; p=0.029, r=-0.23; p<0.001, r=-0.4 and p=0.047, r=0.21, respectively) (Table 3).

**Table 2. Sleep characteristics and serum TWEAK levels in the patient and control groups**

	Patient Group		Control Group	p-value
	Severe OSAS	Mild-Moderate OSAS		
AHI	54.7 (30.3-109.5) <sup>a,b</sup>	9.6 (5-25.6) <sup>c</sup>	2.1 (0.2-4.9)	<0.001
Minimum SPO <sub>2</sub> (%)	79 (43-89) <sup>d,e</sup>	86 (66-92) <sup>f</sup>	87.6 (58-93)	<0.001
TST < 90%	9.7 (0-90.7) <sup>a,b</sup>	0.3 (0-15.4) <sup>a</sup>	0 (0-20)	<0.001
ODI	44.3 (10.8-117.1) <sup>a,b</sup>	7.9 (2.7-23.9) <sup>a</sup>	0.3 (0-3.1)	<0.001
TWEAK (pg/mL)	590.9 (109.2-993.6) <sup>d,e</sup>	797.6 (116.1-998.8) <sup>f</sup>	916.2 (574.8-991.3)	<0.001

All values are median with minimum-maximum range. AHI, apnea-hypopnea index; TST < 90%, time spent with oxygen saturation below 90%; ODI, oxygen desaturation index

<sup>a</sup> Significant higher AHI, TST < 90% and ODI values in severe OSAS patients than healthy controls  
<sup>b</sup> Significant higher AHI, TST < 90% and ODI values in severe OSAS patients than mild-moderate OSAS patients  
<sup>c</sup> Significant higher AHI, TST < 90% and ODI values in mild-moderate OSAS patients than healthy controls  
<sup>d</sup> Significant lower minimum SPO<sub>2</sub> (%) and TWEAK values in severe OSAS patients than healthy controls  
<sup>e</sup> Significant lower minimum SPO<sub>2</sub> (%) and TWEAK values in severe OSAS patients than mild-moderate OSAS patients  
<sup>f</sup> Significant lower minimum SPO<sub>2</sub> (%) and TWEAK values in mild-moderate OSAS patients than healthy controls

**Table 3. Correlation between serum TWEAK levels and sleep-related variables in the patient group**

Variables	p-value	r
AHI	<0.001	-0.41
Minimum SPO <sub>2</sub> (%)	0.047	0.21
TST < 90%	0.029	-0.23
ODI	<0.001	-0.4

AHI, apnea-hypopnea index; TST<90%, time spent with oxygen saturation below 90%; ODI, Oxygen Desaturation Index; r, Correlation Coefficient

## DISCUSSION

There is growing proof of increased inflammation in OSAS. In OSAS, episodic hypoxia-reoxygenation cycles disrupt the oxidative balance and lead to the production of reactive oxygen radicals, which further result in the activation of inflammatory cells (11, 12, 13). Inflammatory cytokines that are synthesized by these activated cells are responsible for the formation of an inflammatory environment and a low-grade inflammation in OSAS. Upper respiratory tract inflammation leads to anatomical narrowing of the upper respiratory tract and an inspiratory pharyngeal muscle dysfunction, thus contributing to the pathophysiological processes underlying OSAS(14). The identification of new inflammatory cytokines for OSAS has been made possible largely by comprehending of the

pathophysiology of this disorder. These new biomarkers identified have the potential to supply knowledge to predict the course of the disease (15). In this perspective, efforts to discover new proinflammatory cytokines in OSAS continue. In this study, in parallel with these efforts, we studied serum levels of a pro-inflammatory cytokine that had not previously been studied in the OSAS patient group.

TWEAK is associated with tumor necrosis factor class and is represented on the exterior of excited immune cells (16). TWEAK has plural function and regulates processes such as growth, differentiation, migration and programmed cell death. Its' pro-inflammatory and pro-angiogenic effects have also been described (17). As its being a multifunctional cytokine, TWEAK levels have been investigated in a wide spectrum of disease groups ranging from chronic inflammatory diseases to autoimmune diseases. TWEAK levels are affected in chronic autoimmune and inflammatory diseases, cardiovascular diseases, renal diseases and malignancies (7,10,18). Patients with chronic autoimmune and inflammatory disease, cardiovascular disease, renal disease and malignancy were not included in our study. In addition, obesity and insulin resistance also affect serum TWEAK levels (6). From this point of view, the clinical and laboratory reflections of obesity and insulin resistance, BMI and HOMA-IR stand out as effective factors on TWEAK levels. In order to evaluate the relationship between OSAS

and TWEAK more accurately, the BMI and HOMA-IR values of the participants were evaluated in this study and no difference was found between these values of the groups.

TWEAK exhibits its' activities including pro-inflammatory response through FGF-inducible molecule 14 (Fn14), a receptor of the TNF family (16). It is known that TWEAK / Fn14 axis plays a role in various pathophysiological conditions. In particular, it is reported that persistent activation of this axis plays a role in pathological cardiovascular remodeling (19). In a review published on this subject, it was emphasized that the treatments targeting this axis promise that it will decrease cardiac dysfunction and decrease the ischemic lesion volume after stroke (19). Another study stated that activation of this axis contributes to skin inflammation (20). In this study, it is emphasized that slight or temporary activation of TWEAK / Fn14 axis contributes to tissue regeneration and repair, but excessive and persistent activation of this axis causes severe inflammation and tissue damage (20). As a result of these effects, TWEAK / Fn14 activation has been reported to play an important role in skin diseases including psoriasis, atopic dermatitis, cutaneous vasculitis, human papilloma virus infection and related skin tumors and cutaneous autoimmune diseases (20). In another study, it was emphasized that overexpression of TWEAK / Fn14 was seen in Crohn's disease and it was suggested to target this axis in the treatment of Crohn's disease (21). The role of the TWEAK / Fn14 axis in the broad spectrum of disease can be explained by the multiple biological activity of this axis.

TWEAK levels increase in chronic inflammatory and autoimmune diseases such as Crohn's disease and psoriasis(7,10,22). However, serum TWEAK levels decrease in conditions that are accompanied by a low-grade inflammation such as obesity, diabetes and insulin resistance(23,24). Increased serum TWEAK levels are not unexpected in chronic inflammatory and autoimmune diseases, but surprising is the decrease of serum TWEAK levels in diseases with low-grade inflammation. Several mechanisms have been proposed to explain these low serum TWEAK levels. According to the most widely accepted explanation, increased expression of FN14 in the target tissue may reduce serum TWEAK levels in the peripheral circulation (25). Therefore, reduction of serum TWEAK levels may be expected in OSAS, to which a low-grade inflammation accompanies. In our study, serum TWEAK levels in the patient group were lower than in the control group. In addition, serum TWEAK levels in the severe OSAS group were lower than in the mild-moderate OSAS group. There was inverse correlation between serum TWEAK levels and AHI was observed.

In some cases of OSAS, systemic inflammation is triggered by chronic intermittent hypoxia. AHI represents the apnea-hypopnea frequencies per hour and might not exhibit the pathophysiological basis of hypoxia in OSAS patients (26). Other polysomnography parameters such as

TST <90% and ODI might be superior to AHI in evaluating the chronic intermittent hypoxia element of the OSAS since they exhibit the frequency and duration of oxygen desaturation (26). When the correlation between sleep-related variables and serum TWEAK levels was examined, there was inversely correlation between TWEAK and TST <90% and ODI, and a positive correlation between TWEAK and minimum SPO<sub>2</sub> (%).

## CONCLUSION

Serum TWEAK levels in OSAS patients and its relation with sleep variables showed for the first time that TWEAK may be a new inflammatory biomarker in OSAS. The main limitation of our study is the small number of patients. There is a need for further studies with larger patient series to determine the importance of TWEAK, a new biomarker that has the potential to show the presence of inflammation in OSAS.

*Competing interests: The authors declare that they have no competing interest.*

*Financial Disclosure: There are no financial supports.*

*Ethical approval: Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (E-16-813).*

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